Degradability of Alfalfa Stem Tissues by Rumen Microorganisms

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Introduction

While alfalfa is recognized as a high-quality forage crop for feeding dairy cattle, the majority of alfalfa's nutritional quality is associated with the leaf fraction. Alfalfa stems contain 60+% cell-wall material and these cell walls are poorly degraded by rumen microbes. Because stems comprise 50% or more of the alfalfa crop, significant improvement in nutrient (energy) availability will require reducing the cell-wall concentration of alfalfa stems or improving cell-wall degradability. The latter approach is complicated by the heterogeneity of tissues that comprise the alfalfa stem. Grabber et al. reported in the USDFRC 1996 Research Summaries that xylem and non-xylem tissues isolated from alfalfa stems were markedly different in their cell-wall degradability. Our objective in this study was to examine differences in stem tissue degradability at the cellular level using microscopy.

Materials and Methods

Alfalfa stems were collected after 31 d of regrowth following cutting in late June 1996. The seventh internode from the base of the stems was excised and preserved in 50% ethanol. Thin sections (100 mm) were prepared from the middle of the internodes using a mirror sectioning procedure. By this method of sectioning the same cell walls which were cut could be examined in two separate sections adhered to slides with double-sided tape. One mirror section from each pair served as a non-degraded control and the other mirror section was incubated in vitro with rumen fluid. Both sets of mirror sections were examined using light microscopy (LM) and scanning electron microscopy (SEM). Degradation of tissues and specific cell-wall layers could be assessed by this procedure. Additional sections were stained with phloroglucinol or ruthenium red to detect the presence of lignin and pectins, respectively. These stained sections were examined by LM.

Results and Discussion

In vitro ruminal degradation resulted in the removal of many alfalfa stem tissues. Figure 1 illustrates the degree of degradation that was observed after 24 h of incubation with rumen microorganisms. Collenchyma, chlorenchyma, secondary phloem, cambium, and protoxylem parenchyma tissues were completely degraded in 24 h (compare Fig. 1a and 1b). Only the cuticle of epidermal tissue remained after degradation (Fig. 1b). None of the completely degradable tissues in alfalfa stems stained positively for lignin. Xylem tissue showed very little degradation and stained intensely with phloroglucinol. Cortical fiber tissue underwent extensive, but incomplete degradation. Figure 1c illustrates cortical fiber cells that had been colonized by rumen bacteria after a 4 h incubation. The thick secondary wall of cortical fibers was still clearly visible after 4 h, but after 24 h of incubation all secondary wall material had been removed from the cortical fiber cells (Fig. 1d). Only a thin primary wall remained undegraded. The primary walls of cortical fibers were observed to be thickened when viewed by LM, but these primary walls shrank in thickness due to the dehydration procedure needed to prepare sections for SEM. Under LM it was observed that the thick primary walls stained strongly for pectins, but only the luminal edge of the primary wall stained positively for the presence of lignin. The secondary wall of cortical fibers did not stain for either pectins or lignin. The use of LM revealed that the thick, non-lignified primary wall of cortical fibers was completely degraded leaving only the thin, lignified primary wall structure seen in Figure 1d.

Conclusions

Alfalfa stems contain tissues which are completely degradable, virtually undegradable, and partially degradable by rumen microbes. The presence of lignin

is a good indicator of tissues which are poorly degraded. Contrary to previous reports in the literature, all secondary wall material is not lignified as evidenced by the alfalfa cortical fiber secondary cell walls. The results of this research suggest two routes to improving alfalfa stem cell-wall degradability. One possibility would be to prevent or reduce cell-wall lignification of tissues which normally lignify. The alternative approach would be to increase the proportion of the stem comprised of those tissues which are completely degradable. The successful path to improving alfalfa stem degradation will depend on which modifications yield an agronomically viable crop.

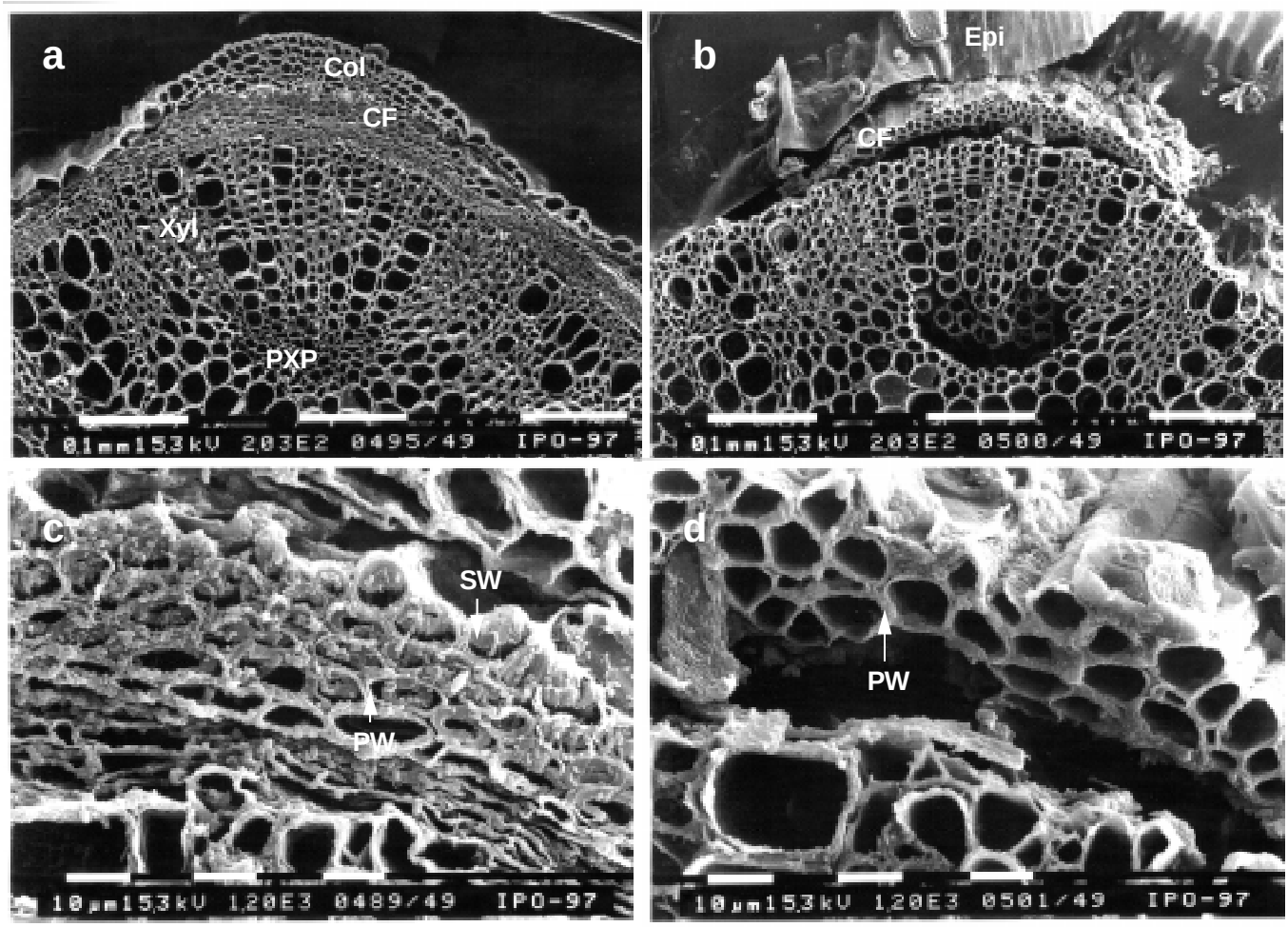

Figure 1. Scanning electron micrographs of alfalfa stem tissues before (a) and after (b) in vitro degradation by rumen microorganisms for 24 h. Collenchyma (Col), epidermis (Epi), and protoxylem parenchyma (PXP) were completely degraded, whereas cortical fiber and xylem were only partially degraded. Extensive bacterial colonization of thick secondary walls (SW) of cortical fiber was observed after 4 h of incubation (c) and only the primary wall (PW) remained after 24 h of degradation (d).